Studies on the Infection of Cucumber Mosaic Virus V. The Observation of Epidermal Cell in the Local Lesion

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Studies on the Infection of Cucumber Mosaic Virus
V. The Observation of Epidermal Cell in the Local Lesion

Yoshio EHARA and Tadao MISAWA

Department of Agronomy, Faculty of Agriculture*
Tohoku University, Sendai, Japan
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Summary

On the combination of CMV and cowpea, the relation between local lesion formation and epidermal cell was observed as follows:

1) When the inoculated epidermis of the cowpea was separated from the leaf before the occurrence of local lesions and cultured, local lesions did not form on these epidermis. On the other hand, local lesions were formed in the uncovered residual mesophyll tissue.

2) All epidermal cells in the local lesion died after the occurence of necrosis in the mesophyll tissue.

3) The necrosis of the epidermis in the local lesion would be caused by the influence of the necrosis of mesophyll tissue.

4) The primary infected cells were of two types. One type is the cells which died before the occurrence of local lesion, and the other is the cells which were alive until the occurrence of local lesion. These differences would be caused by the degree of cell injury at the inoculation.

5) In order for infected cells to increase, the primary infected cell must be alive until the infective entity produced in the primary infected cell has spread to adjacent cells.

When tobacco hair cells were inoculated with tobacco mosaic virus (TMV) by the micro-injection method, the multiplication of TMV was evident (1). It has been accepted generally that plant virus is able to multiply in the epidermal cell of host plants. But with the combination of CMV and cowpea leaf, it was thought that the virus did not multiply in the epidermal cell but in the mesophyll cell, because the virus multiplication in the epidermal cell was not evident under bioassay (2). In this case, the primary infected cell is the epidermal cell, and the earliest infection processes of the inoculated virus such as decoating and other changes would be performed in this cell. Therefore, it was presummed that the epidermal cell was a necessary site for the infection, even if CMV did not multiply there (3).

* Laboratory of Phytopathology

Until now, there are very few reports on primary infected cells. Concerning the virus entrance into the host, Brant presummed the ectodesmata to be the pathway from the cuticle to the epdiermal cytoplasm (4). On the other hand, Kontax and Schlegel (5), Herride and Schlegel (6) thought that virus would enter from the besal septa of hair cells which were borken by rubbing inoculation. Our experimental plant, i.e. cowpea primary leaves have no hair cells, so that when the primary leaves are rubbed with the virus, the primary infected cells are the epidermal cells.

In this experiment, using CMV on cowpea leaves, the formation of the necrosis of epidermal cells and the changes in primary infected cells were observed.

Materials and Methods

Virus and Plant

The virus used was the ordinary strain of cucumber mosaic virus (CMV-O). The virus was multiplied on tobacco leaves (bright yellow) and purified by the method of Soctt (7). The plants used were cowpeas (*Vigna sinensis* Endle var. Kurodane Sanjaku) which form necrotic local lesions. The cowpeas and tobacco plants were grown in a growth cabinet (8). The primary leaves of cowpea were used on the seventh to ninth day after sowing. After the inoculation, the inoculated cowpea leaves were floated on Knop's solution in a covered petri dish, and incubated under continuous fluorescent light of 3000 lux at 28°C.

Inoculation

The inoculum was made by suspending the purified virus in 0.1 M phosphate buffer (pH 7.0.). Inoculation was done by the carborundum method (8).

Microscopic observation

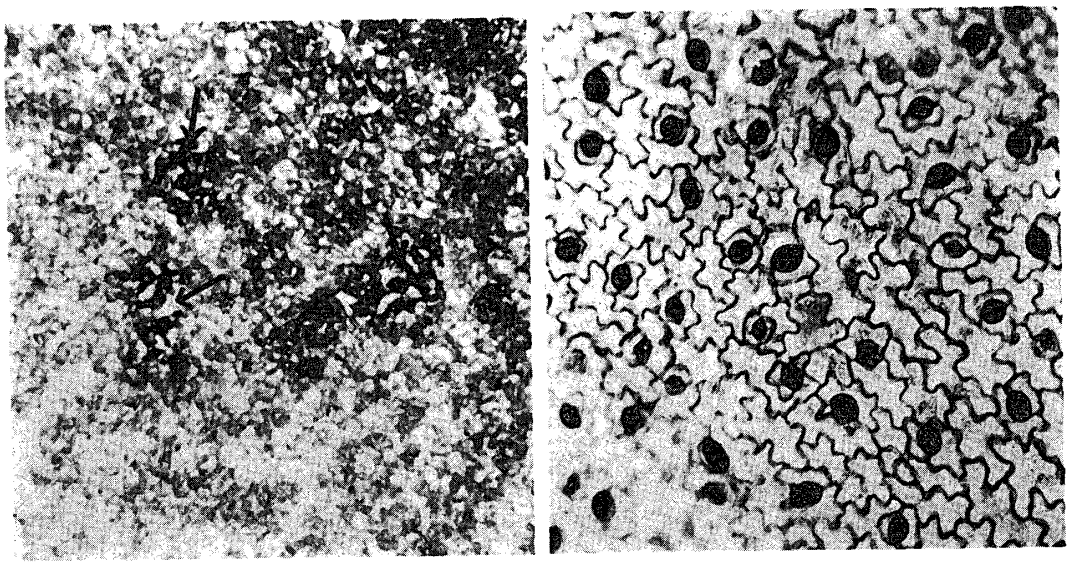
Fresh tissues were stained by 0.005% neutral red, 0.01% orange G, 0.01% safranine, 0.001% methyl red and pyronine methyl green, and observed by microscopy. As dead cells are stained deeply by these dyes as compared with living cells, the living and dead cells could be distinguished easily. The tissue fixed in ten minutes by Formalin-Alchol-Acetic acid (F.A.A.) was stained by 0.01% safranin and pyronine methyl green.

Results

Relation Between the Formation of Local Lesion and Epidermal Ccell

In this experiment, whether the local lesion is formed or not in the epidermal tissue which has been separated from the leaf, was observed in order to clarify the relation between the formation of local lesions and epidermal cell. The lower epidermis of the cowpea primary leaf was inoculated with CMV. At 2–3 hours after the inoculation, by this time the infective entity had spread already from the epidermis to the mesophyll cell as described in the previous reprot (3), the in-

oculated epidermal tissue was stripped. Both the stripped epidermis and the leaf tissue from which it was removed were cultured in Knop's solution for 24 hours, because local lesions were formed in the untreated cultured leaves used as a control during those 24 hours. Then the epidermal tissues were stained by neutral red, orange G or safranine, for dead cells should be stained deeply by these dyes. However, as cells stained deeply were not observed, it was confirmed that dead cells did not exist in these epidermal tissues. That is, dead and necrotic cells are not formed in the epidermal tissue which were removed from the leaf. On the contrary, the necrotic cells were formed in the mesophyll tissue from which the epidermis was removed at 2–3 hours after inoculation (Fig. 1).



(A) (B)

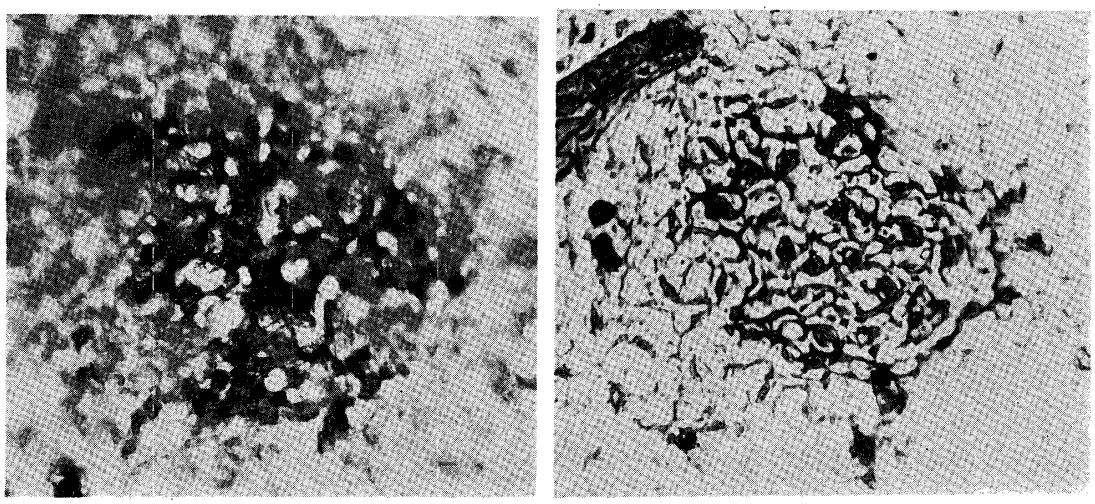
FIG. 1. (A) Local lesions formed in the mesophyll tissue where the inoculated epidermis has been stripped (arrow).
(B) Stripped and cultured epidermis in which the local lesions are not observed.

Epidermal Cells in the Local Lesion

After the occurrence of the local lesion, the epidermis in the area of the local lesion was stripped and observed by a microscope. All epidermal cells corresponding to the portion of the necrotic mesophyll cell had died and browned slightly. When these epidermal cells were stained with safranin or pyronine methyl green after having been fixed with F.A.A., these cells were stained in deep red. Moreover, the necrotic area of the epidermis was slightly larger compared to that in the mesophyll tissue (Fig. 2).

The Change of the Primary Infected Cell

The changes in the primary infected cells were observed. In this case, as the inoculation was done to the epidermis, the primary infected cells were the epidermal

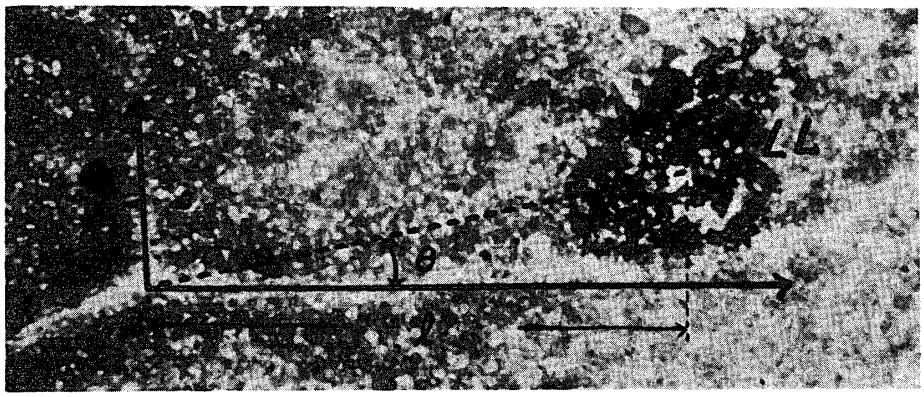


(A) (B)

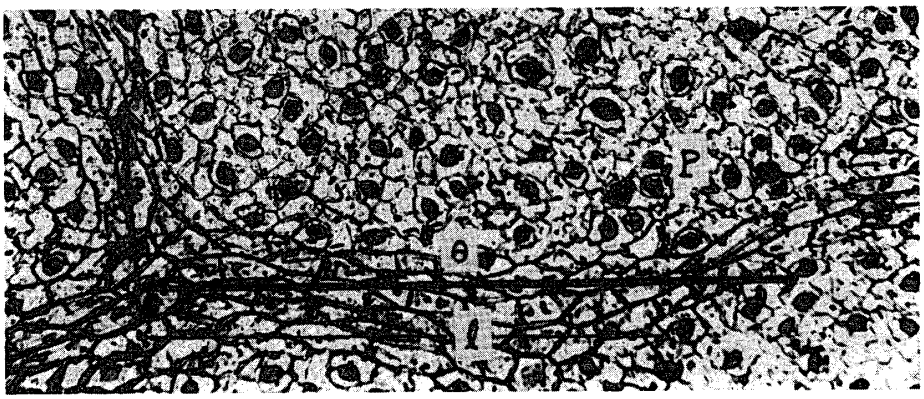
FIG. 2. (A) Mesophyll of a local lesion area where the epidermis has been removed. (B) Stripped epidermis of a local lesion area.

cells. After the appearance of necrosis, the primary infected epidermal cell could not be distinguished from other epidermal cells in the necrotic lesion. Therefore, it is necessary that the observation of the primary infected cells be done before necrosis occur in epidermis. When the inoculated epidermal tissue was separated from leaf after the inoculation, necrotic cells did not occur in these epidermal tissues. Therefore, the inoculated epidermis was stripped before the occurrence of local lesion and both the stripped epidermis and uncovered residual mesophyll tissue were cultured. Then after the occurrence of local lesion in the cultured mesophyll tissue, the portion of the stripped epidermis corresponding to the portion of the local lesion formed in the uncovered mesophyll tissue, was found from the positional relation to the vein in the vicinity of the lesion as shown in Fig. 3. Morphological changes of the epidermal cell in these portions were microscopically observed. Staining of the tissue was done by the above-cited dyes.

The plasmolyzed and coagulated cells were frequently observed in the center in these portions. Their protoplasm was stained well by neutral red and in pink color by pyronine methyl green (Fig. 4). Therefore, it was judged that these cells were dead. The circlic portion (R) on the surface of the epidermis would be wound portion which was inflicted by rubbing with carboundum at the time of inoculation. These cells would have died after the infective entity migrated to the adjacent cells, for the degree of injury to the cells was comparatively small. The cells which surrounded this dead cells could not be distinguished from other cells in either internal morphology or color tone by the stain. But such dead epidermal cells did not exist in the epidermis at every local lesion. Also, it was observed that every epidermal cell did not change in stainability or morphology, compared with healthy cells. From these observations, it was found that there were two types



(A)



(B)

FIG. 3. (A) A local lesion which occured in the uncovered mesophyll tissue (LL). This position was found by the vein as a base line (distance (l), angle (θ), was measured) (B) The inoculated site (P) in the epidermis which corresponded to the local lesion of the mesophyll (A) was decided by l and θ.

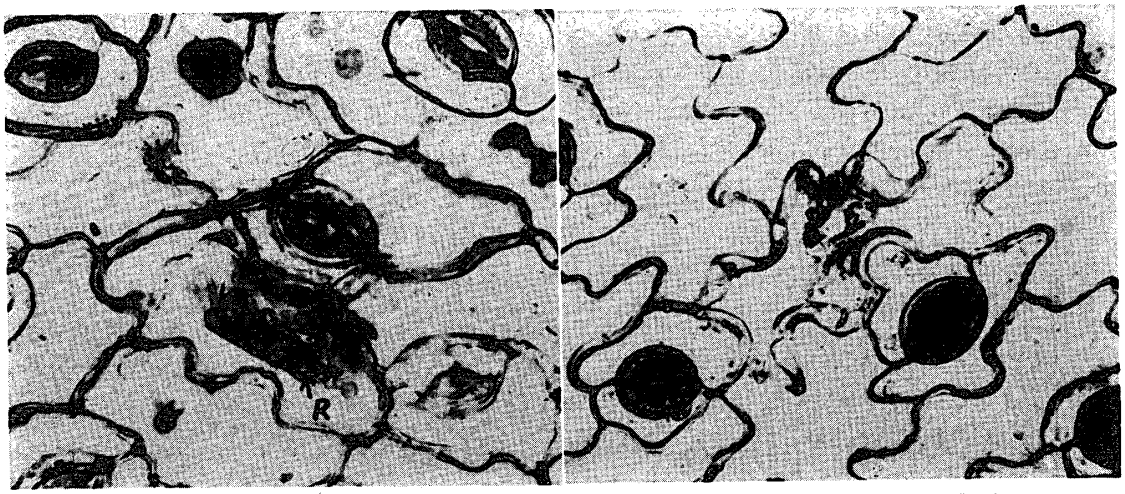


FIG. 4. The morphological change of the primary infected cell. plasmolysis and coagulation of protoplasm were recognized. R: Injured portion. C: Coagulated protoplasm.

of morphological changes in the primary infected epidermal cell. One type is the cell which died before the occurrence of the necrosis and after the infective entity migrated to the under mesophyll cell. The other type is those which were living until the occurrence of necrosis in the mesophyll tissue. This difference would be caused by the degree of injury that the cell received. Moreover, local lesions did not completely develope when the epidermal cells were injured severely (Fig. 5).

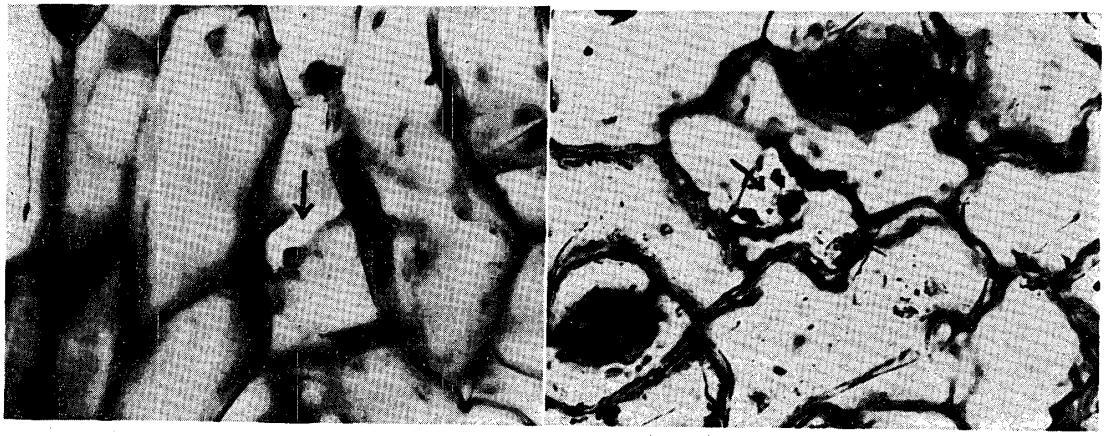


FIG. 5. Epidermal cell wall which received sever cleaving by inoculation (arrow). Local lesion did not occur in this area. Arrowed triangular area is cleaved part of the epidermis.

These observations indicate that when the primary infected cell dies immediately for a severe injury, or when this cell dies before the infective entity spreads to the mesophyll cells, the infection is prevented and a local lesion does not appear.

Discussion

In this experiment, the local lesions were not formed in the cowpea epidermal tissue which was removed from the leaf at 2–3 hours after being inoculated and cultured. But when the epidermal tissue in the local lesion was observed after the occurrence of the local lesion, these epidermal cells were necrotic. On the other hand, the virus activity in cowpea epidermal tissue inoculated with cucumber mosaic virus could not be recognized as described in report IV (2). From the above results, on the combination of CMV and cowpea leaf, the portion where the virus multiplies and consequently the cells die as the direct effect of the virus would be mesophyll cells. Therefore, it is supposed that necrosis of the epidermal cells in the area of the local lesion would not be caused by the direct effect of virus replication, but by the indirect influence of the necrosis of the mesophyll cells. This indicates that necrosis is generated from the mesophyll cells. Whereas, it is presummed that the epidermal cell has a very important role as the area where the virus introduced by the inoculation undergoes

decoating, etc. as described in Report III (3).

There were two types of primary infected epidermal cells. One type is the cell which dies a few hours before necrosis occurs in the mesophyll tissue, and another is the cell which is still alive until the occurrence of local lesion. Such types would be caused by the degree of cell injury inflicted when rubbing with carborundum for inoculation. It was presummed that the inoculated virus would decoat in the primary infected cell as described in Report III (3). Therefore, in the case of dead cells before the occurrence of local lesion, the infective entity produced there would have migrated into the adjacent cells before the cell died.

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